

**REMARKS**

This Supplemental Amendment supplements the Amendment filed June 8, 2010, and is also filed in reply to the Office Action dated November 29, 2009, is believed to be fully responsive to each point of objection and rejection raised therein. Accordingly, favorable reconsideration on the merits is respectfully requested.

Claims 47-55, 57, 59-60, 63-79, 83-84, 86-89, 91-98, and 101-123 are all the claims pending in the application. Claims 1-46 are canceled. Claims 48-55, 57, 59-60, 63, 67, 69-79, 83-84, 86-89 and 92-98 are withdrawn from consideration. Claims 115-123 are newly added. Exemplary support for new claim 115 may be found throughout the specification, at for example Page 4 line 27 to Page 5 line 18 and Page 7, line 25-28 and Page 25, line 28 and Page 27, lines 4-7 and 29-33. Exemplary support for new claim 116 may be found throughout the specification, at for example Page 25, line 31 to Page 26, line 3. Exemplary support for new claim 117 may be found throughout the specification, at for example Page 26, lines 1-3. Exemplary support for new claim 118 may be found throughout the specification, at for example Page 45, lines 1-4. Exemplary support for new claim 119 may be found throughout the specification, at for example Page 26, lines 1-3 and Page 45, lines 1-4. Exemplary support for new claim 120 may be found throughout the specification, at for example Page 44, lines 10-11 and Page 42, lines 9-13. Exemplary support for new claim 121 may be found throughout the specification, at for example Page 44, lines 10-11 and Page 42, lines 9-13 and Page 39, line 12 to Page 40, line 3. Exemplary support for new claim 122 may be found throughout the specification, at for example Page 40, lines 4-13. Exemplary support for new claim 123 may be found throughout the specification, at for example Page 39, line 12 to Page 40, line 3. Exemplary support for new claim 124 may be found throughout the specification, at for example Page 40, lines 4-13. No new matter is added

by way of this amendment. Entry and consideration of this amendment are respectfully requested.

Applicants respectfully request the Examiner to consider the arguments presented in the Amendment filed June 8, 2010. Additionally, Applicants submit the following additional remarks in response to the rejection under 35 U.S.C. § 103(a) over McCrea (Molecular Pharmacology V. 49, pp. 927-937 (1996)) (“McCrea”) in combination with U.S. Patent No. 6,171,794 to Burchard (“Burchard”).

In particular, Claims 115 to 121 recite the preferred red emitting dyes, having high quantum yield fluorescence. These dyes are particularly suited for binding to and visualization of GPCR receptors, whereby the fluorescence is distinct from both cell autofluorescence and, where present, fluorescence from GFP tagged receptors.

Claims 117 to 119 recite an unexpected feature of the invention whereby only the GPCR-bound fluorescent ligand is visible, the unbound ligand showing low background fluorescence whereby it is not visible. This has the particular advantage as claimed that unbound ligand need not be washed away before visualization. Accordingly, the results can be considered as highly accurate, as there is less risk that bound ligand has been dissociated during washing, moreover as described at page 45, lines 16-20, it is possible to measure fluorescence in real time. (“With ligands showing low background fluorescence it is not necessary to remove unbound ligand by washing before performing either confocal microscopy or FCS. It is therefore possible to measure fluorescence with time, in both time and concentration dependent manner.”)

In the case of BODIPY 630/650, the fluorophore FI appears to embed in the cell membrane region in manner that the fluorescence is intensified, in contrast to fluorophore remaining in aqueous phase, outside the cell membrane. This is described and illustrated in the

figures of the present application and in the publication by the inventors, Baker et al., *British Journal of Pharmacology*, vol. 159:772– 86 (2010)(copy submitted herewith).

In the figures of the present application, the cell membrane incorporating receptors is clearly illustrated. The specification, at Page 63 lines 25-31 and Page 66 line 7-10, describes the binding taking place over a period of up to 60 minutes incubation, with visualization at 5 and 30 minutes, i.e during the incubation period, and importantly therefore still in the presence of unbound fluorescent ligand.

Furthermore, Baker et al. (2010), Figure 7 also shows good visualization of binding for BODIPY 630/650 (A, B, C, D). However, when the Texas Red fluorophore was used, during incubation the entire field was red and visualization was not possible, only after washing away unbound ligand (E and F), good visualization of binding was achieved. The dansyl class of dyes are also capable of giving clear real time visualization of bound fluorescent ligand if the dansyl component protrudes from the membrane region where it is quenched/bleached in the extracellular aqueous region and is thus not visible. Referring to Figure 8 in Baker et al (2010), this effect is shown, by imaging the fluorescent ligand in two media, mimicking the cell membrane phase (MeOH) or the aqueous medium (HBS). In the case of Texas Red (B) these are not distinguishable, however in the case of BODIPY 630/650, visualization is only possible in MeOH. In the case of dansyl, visualization is possible in both phases, but in MeOH it is short-lived.

Figure 9B shows further proof of visualization of ligand binding of XAC-X-BODIPY 630/650. Black dye quenches anything fluorescing in the aqueous environment. As there is no difference between A and B it is clear that the fluorescent ligand does not fluoresce in the aqueous environment.

**SUPPLEMENTAL AMENDMENT UNDER 37 C.F.R. § 1.114(c)**  
**U.S. Application No. 10/551,475 (Q111431)**

Accordingly, Applicants respectfully request withdrawal of the section 103 rejection. Entry of this amendment and allowance of the application are respectfully requested.

**II. Conclusion**

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The U.S. Patent and Trademark Office is hereby directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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**23373**

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